THE PRE-GRAVID PHASE OF OVARIAN DEVELOPMENT
IN ANOPHELES FUNESTUS*

BY

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In a study of ovarian development in Anopheles gambiae (Gillies, 1954a), it was found that gonotrophic discordance—a term used to indicate the need for multiple blood-meals in the course of a single gonotrophic cycle—occurred in about 20 per cent. of wild-caught females. If dissection of freshly fed females was delayed until digestion of the blood-meal was nearly complete, the population could be divided into two groups: those in which development of the ovaries had proceeded normally to Christopher's (1911) stage V, and those in which it had not advanced beyond stage II. The former group was therefore composed of gravid females, and to the latter the term 'pre-gravid' was applied. The pre-gravid group was shown to be composed mainly of new emergences. It was concluded that, owing to the undeveloped state of the ovaries in virgin females (Christopher's stage I), a single blood-meal was not sufficient for full maturation of the ovaries, and that at least two feeds were required for the production of the first egg batch. The first gonotrophic cycle could, therefore, be divided into a preliminary pre-gravid phase and, following the second blood-meal, a secondary gravid phase of development. In this way, females in which development was completed after a single feed in all except a very small minority. This physiological characteristic made it possible to recognize the youngest age-group amongst recently fed mosquitoes, and it was shown that useful information on the age composition of the population could be obtained by the method.

In the course of the work on A. gambiae, exactly similar observations were made on A. funestus. In the present paper the results of these studies are recorded, in so far as they indicate (i) the extent to which multiple blood-meals are required by A. funestus in the course of a single cycle, (ii) the proportion of such females found to be newly emerged, and (iii) whether the recognition of the young pre-gravid group can be used to provide information on the age composition of populations of this species.

An account has been given elsewhere of the low-lying coastal area of Tanganyika chosen for these studies (Gillies, 1954b). The technique employed in the present work was the same as that described for the investigation of A. gambiae; that is to say, gorged females were collected from experimental huts or from ordinary African huts, they were kept for 24 hours before being killed, and, after examination with a hand-lens, the gravid and pre-gravid groups were separated on the basis of the external appearances. The proportion of the pre-gravid group of females in the whole recently fed population is recorded as the pre-gravid rate.

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THE PRE-GRAVID RATE IN *A. FUNESTUS*

In Table I the results, expressed as monthly arithmetic means, are shown of a series of daily hand-catches of *A. funestus* made over a 12-month period in an experimental hut. The mean of 13 four-weekly averages gives a pre-gravid rate of 20.7 per cent., or 24.1 per cent. of the actual total of mosquitoes examined. This shows that in rather more than 20 per cent. of females multiple blood-meals are required in the course of a single gonotrophic cycle.

The pre-gravid group was further examined in order to determine to what extent it was composed of newly emerged females. Dissection of the reproductive tract, with particular reference to the fertilization index and the condition of the oviducts and their ampullae, gave the following results: (i) of 1,636 pre-gravid females dissected, 65.5 per cent. were fertilized; (ii) of nearly 500 fertilized pre-gravidas examined, 73 per cent. were found to be nulliparous, 20 per cent. were multiparous, and 7 per cent. were of doubtful age.

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<tbody>
<tr>
<td>Total no. caught</td>
<td>....</td>
<td>1,129</td>
<td>660</td>
<td>773</td>
<td>472</td>
<td>417</td>
<td>278</td>
<td>256</td>
<td>707</td>
<td>626</td>
<td>633</td>
<td>366</td>
<td>48</td>
<td>16</td>
</tr>
<tr>
<td>% pre-gravid</td>
<td>....</td>
<td>323</td>
<td>282</td>
<td>153</td>
<td>107</td>
<td>90</td>
<td>56</td>
<td>58</td>
<td>204</td>
<td>160</td>
<td>94</td>
<td>75</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Fertile pre-gravid</td>
<td>....</td>
<td>29</td>
<td>29'4</td>
<td>10'4</td>
<td>22'7</td>
<td>21'6</td>
<td>20'1</td>
<td>22'7</td>
<td>28'8</td>
<td>25'3</td>
<td>14'8</td>
<td>20'5</td>
<td>2'1</td>
<td>12'3</td>
</tr>
<tr>
<td>Average no. biting per night</td>
<td>....</td>
<td>37'3</td>
<td>31'2</td>
<td>36'1</td>
<td>21'5</td>
<td>17'5</td>
<td>12'1</td>
<td>10'8</td>
<td>31'3</td>
<td>28</td>
<td>31'8</td>
<td>15'6</td>
<td>2</td>
<td>0'7</td>
</tr>
</tbody>
</table>

The characters used for the recognition of nulliparous females were those that can be observed when dissection is made in normal saline, namely: (a) the ampullae are cream-coloured rather than yellow, translucent, and only slightly darker than the oviducts, with transverse folds slight or absent; they are indistinctly separated from one another, but more clearly so from the common oviduct; (b) the paired oviducts are slender, their junction with the ampullae clearly demarcated and the upper ends narrow; and (c) the lumen of the ovaries is not patent.

Thus it may be concluded that about 16 per cent. (i.e., one quarter of the 65.5 per cent. fertilized) of the pre-gravid group are old multiparous females, while the great majority are young nulliparous. The pre-gravid group in *A. funestus* is therefore, as in *A. gambiae*, predominantly composed of new emergences.

It was not possible by this method of study to ascertain what proportion, if any, of newly emerged females took only a single blood-meal during the first gonotrophic cycle; in other words, what proportion might by-pass the pre-gravid phase under natural conditions. But, in a small series of observations on unfertilized females bred from larvae, it was noted that, in six out of 24 mosquitoes which were deprived of sugar-water and given a single blood-meal, development of a proportion of the follicles proceeded to stage V. The possibility exists that this may also occur to some extent in nature, and that some newly emerged females may exist unrecognized within the gravid group.
COMPARISON OF THE PRE-GRAVID PHASE IN *A. FUNESTUS* AND *A. Gambiae*

Table II has been so constructed as to make possible comparison of various aspects of the pre-gravid phase in *A. funestus* and in *A. gambiae*. The catches were made in an experimental hut over the same 12-month period and under the same conditions for both species. The results show that there is little difference in the numbers requiring multiple blood-meals in the course of each cycle (Table II, a). Among the *A. funestus* females, however, a much higher proportion of pre-gravids is fertilized than among *A. gambiae* (Table II, b). In both species most of these fertilized pre-gravids are new emergences (Table II, c), but, since such fertilized females are much commoner in *A. funestus* than in *A. gambiae*, it follows that gonotrophic discordance among old females is nearly twice as frequent in *A. funestus* (Table II, d and e). Thus the pre-gravid rate in *A. funestus* is correspondingly less precise a measure of the incidence of new emergences in the biting population. Nevertheless, it will be seen that removal of the multiparous element from among the pre-gravids (Table II, a-e), in order to give a corrected and truer picture of the size of the newly emerged group, reduces the index only from 20.7 to 17 per cent., and does not materially affect its usefulness as a method of detecting young females in populations of this species.

To make these points clearer, the pattern of ovarian development in both species is summarized diagrammatically in fig. 1.

**Table II**

Comparison of the pre-gravid phase in *A. funestus* and *A. gambiae*

<table>
<thead>
<tr>
<th>Description</th>
<th><em>A. funestus</em></th>
<th><em>A. gambiae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean monthly pre-gravid rate</td>
<td>20.7</td>
<td>19.6</td>
</tr>
<tr>
<td>Fertilization index of pre-gravids</td>
<td>65.5</td>
<td>56.1</td>
</tr>
<tr>
<td>Percentage of fertilized pre-gravids found to be multiparous</td>
<td>20.27</td>
<td>20.32</td>
</tr>
<tr>
<td>Percentage of all pre-gravids found to be multiparous</td>
<td>13.17</td>
<td>7.58</td>
</tr>
<tr>
<td>Percentage of all females multiparous and showing gonotrophic discordance</td>
<td>3.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Corrected percentage of new emergences in whole population</td>
<td>17</td>
<td>17.8</td>
</tr>
</tbody>
</table>

**AGE COMPOSITION OF *A. FUNESTUS* POPULATIONS**

In order to study the relationship of the pre-gravid rate to changes in population density, monthly values of the pre-gravid rate and of the biting rate in an experimental hut are set out graphically in fig. 2. It is apparent from the figure that, when populations are very large or very small, the pre-gravid rate is correspondingly greater or lesser; at middle ranges the correspondence is slight. Analysis of this correlation by the use of Kendall's ranking coefficient gives a value of $\tau$ lying between 0.31 and 0.49 = 0.21. This exceeds the 5 per cent. level of significance, but the margin is not great. The low level of
significant correlation, and the general shape of the curves in fig. 2, suggest that it is only
near the upper and lower limits of population density that the rate of change is sufficiently
striking to affect the proportion of new emergences as evidenced by the pre-gravid rate.
The long decline during the dry period lasting from week 9 to week 24 was evidently too
gradual for any major changes in age composition to be apparent from either the pre-gravid
rate or the sporozoite rate. As already reported (Gillies, 1954a), the sporozoite rate in

ANOPHELES FUNESTUS.

![Diagram of Anopheles Funestus life cycle]

ANOPHELES GAMBIAE.

![Diagram of Anopheles Gamia life cycle]

Fig. 1. Diagrams showing the successive phases of gonotrophic activity observed in natural populations
of A. funestus and A. gambiae in the East African coastal lowlands.

A. funestus in the area of Tanganyika where this study was carried out was found to be
relatively stable. During the period covered by weeks 1-34 the monthly rate in gravid
females remained within the range of 1-5-3-3 per cent. Unfortunately the number of
collections during the period of rapid population decline, when the sporozoite rate might
have been expected to rise, was too small to be of value.
The general picture of age composition in the *A. funestus* populations studied, given by this analysis, is one of stability. If the weekly fluctuations in the pre-gravid rate and the biting rate are studied, the changes in both are far less abrupt than those observed in *A. gambiae*, in which species they were regarded as indicative of sudden outbursts of breeding activity. From this it may be inferred that changes in the production of adults from breeding-sites are less striking in *A. funestus*. In view of the semi-permanent nature of many *A. funestus* breeding-sites, this is not an unexpected finding.

![Graph showing monthly variation in the pre-gravid rate and biting rate of *A. funestus* in an experimental hut.](image)

**DISCUSSION**

It is clear from the results recorded in this paper that pre-gravid females are as common in populations of *A. funestus* as in *A. gambiae*, and that in *A. funestus*, as in *A. gambiae*, they are essentially a newly emerged group. Macdonald (1952) suggested that it should be possible to make deductions as to the mortality rate of a species by observing the proportion of nulliparous females in the population. Following up this suggestion, Davidson (1954) made use of the method in order to estimate mortality rates for *A. gambiae* in different parts of East Africa. In considering the data presented here, it seems to the present author that it would be legitimate to compare the pre-gravid rate in *A. funestus* at the same place under different conditions, and that, if observations were continued over an adequate period of time, it might be possible to draw valid conclusions as to the relative mortality rates under the two sets of conditions. But it is by no means certain that a pre-gravid
ase is essential for all *A. funestus* females. For that reason, although the pre-gravid

e is much the same in both species, it would be unwise to assume that the biting

dulation must contain the same proportion of new emergences in both, and that therefore

vitality rates in the area where the present studies were carried out must be similar.

Further work on *A. gambiae* has indicated that, under the very different ecological

ditions existing in a mainly arid inland region of Tanganyika, two blood-meals are not

essarily taken by all females in the course of the first gonotrophic cycle. Much work

before remains to be done. It is not clear, for instance, whether the pre-gravid phase is a

acteristic of all strains of *A. gambiae* and is only modifiable by the environment, or

ther the genotype may vary as well. But it seems possible that a pre-gravid phase

exist in other anophelines, as it does in *A. gambiae* and *A. funestus*, and that it may

ound under some circumstances to provide useful information about the age composition

be mosquito populations studied. In the present instance, it has served to illustrate

relative stability of established *A. funestus* populations, and suggests that, during

ods of build-up and of abrupt decline, predominantly young and old populations

ctively can be recognized.

SUMMARY

1. In a study of ovarian development in natural populations of *Anopheles funestus* in

Africa, it was found that 30.7 per cent. of fed females required more than one blood-

for maturation of the ovaries.

2. Among this group, 34.5 per cent. were unfertilized, while, of those fertilized, 73

ent. were found to be nulliparous. These females were therefore mainly new-

ences.

3. It is concluded that a pre-gravid phase of development, in which the ovaries do

advance beyond Christopher's stage II, follows the first blood-meal in newly emerged

estus females. The composition of the pre-gravid group differs in certain respects

that previously found in *A. gambiae*. In both species this character can be used for

cognition of the newly emerged element in wild populations.

4. Comparison of monthly variation in the proportion of newly emerged females with

al numbers caught biting in an experimental hut indicates the relative stability of the

osition of populations of *A. funestus*.

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p. of the malaria assistants, A. J. Osumbah and F. J. Ojikii, is also recorded with

REFERENCES


74, 702.

w. W. (1932). The recognition of age-groups within populations of *Anopheles gambiae* by the pre-gravid


w. W. (1954). Studies of house leaving and outside resting of *Anopheles gambiae* Giles and *Anopheles

